Radiat. Phys. Chem. Vol. 14, pp.639-653. 0020-7055/79/0901-0639 $02.00/0

FACTORS AFFECTING RADIOLYTIC EFFECTS IN FOOD

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ABSTRACT

On basis of radiation chemical considerations supported by model system studies, several general predictions can be made regarding the influence of specific parameters on the chemical effects in irradiated foods. Among the parameters considered are: food composition, physical state of components, irradiation temperature, dose-rate, and total dose. Composition determines the type and extent of reactions initiated by both direct and indirect radiolytic interaction. The multiphasic character of foods influences cross reactions between components. State of hydration introduces both physical and chemical aspects into these considerations. Irradiating systems in which the water is frozen changes substantially the chemical effects because the diffusion of reactive entities is impeded. Temperature, especially in frozen systems, has a specific effect on the type and extent of reactions that could occur. Dose and dose-rate, insofar as they affect the pathways for reaction of intermediate species with the original or new components, also determine the type and amount of final radiolysis products. Illustrations of many of these features of the chemistry have been drawn from experiments on model systems of proteins, frozen aqueous solutions, and compounds derived from lipids, as well as from irradiated meats and poultry. These considerations and related experimental results explain the validity of using chemical data to obtain clearances and to extend clearances from approved foods to other foods or to other irradiation conditions.

INTRODUCTION

There are several factors relating to the food to be irradiated and to the irradiation process that determine the chemical reactions that will take place in the food. Composition is a particularly important factor, since it determines the spectrum of radiolysis compounds produced. Physical state, which includes whether the system is rigid or fluid and whether it is mono- or multiphasic, influences the yields of the individual products. Other processing factors such as temperature of irradiation, dose-rate, and total dose affect the pathways for reaction, further affecting the yields. A knowledge of the reactions leading to these products and how they are affected makes it possible to predict the types and levels of radiolysis products.

Ultimately, the consideration of the safety of irradiated food amounts to evaluating the physiological significance of consuming foods containing low levels of protein, lipid, and carbohydrate degradation products. Putting these compounds into perspective with regard to their nature and amounts is critical and helpful to health authorities involved in approving the use of such foods. Such information should and will be submitted along with toxicological and microbiological data when

petitioning for clearing irradiated foods or for extending existing clearances to other, similar foods or to foods irradiated at other related conditions. The approach of using chemical data for obtaining clearances would eventually supersede relying upon cumbersome animal feeding studies, once experience with it has been gained.

To give an overview of how the chemistry data should be viewed and could be used, this paper considers how composition, phase, temperature, dose-rate, and dose affect the product yields. It provides a basis, using a chemical approach, for predicting whether foods and/or specific processes are similar and for indicating the validity of extrapolating conclusions on approved foods to other foods. For illustrative purposes, results from model systems and actual foods will be presented. Specific proteins, frozen aqueous systems, and lipids were used as models; while the actual foods used included various meats, poultry, and fish items.

EXPERIMENTAL

Most of the procedures used in acquiring the illustrative data have been or will be described elsewhere. Special features of the procedures or equipment for the electron spin resonance, electrophoretic, gas chromatographic/mass spectrometric, and electrochemical analyses will be summarized briefly.

Electron Spin Resonance (ESR)

Measurements of free radicals in proteins or foods have been made using a Bruker 420R ESR spectrometer (1,2). Samples of food were prepared by blending with added water, making a slurry, and then forming frozen sticks (3 mm x 15 mm) for irradiation and ESR examination at −80°C. Standard scanning and modulation techniques were used for the samples in the Bruker universal resonator and the Micronow special resonator for *in situ* irradiation. Temperature was controlled to ± 1°C using the Bruker low temperature sample holder and controller.

Electrophoresis

Electrophoretograms of the proteins in beef, pork, ham, and chicken were obtained using the lipid-free fractions extracted with 8 *M* urea in the presence of a reducing agent, either dithiothreitol (DTT) or β-mercaptoethanol. Electrophoresis was performed using 5% polyacrylamide gels in the presence of SDS (3). Staining was done with Coomassie blue. Densitometric recordings were done using a Gilford gel scanner-spectrophotometer system.

Gas Chromatography and Mass Spectrometry (GC/MS)

The analyses of radiolysis products from various meats (beef, pork, ham, lamb, mutton, veal, and chicken) were performed using combined gas chromatography and mass spectrometry (4,5). Many of the compounds formed are volatile and may be analyzed directly by GC/MS after distillation from the meat using high vacuum (10^{-3} Torr) techniques. In the case of less volatile compounds, the meat was extracted with a solvent for lipids and the products separated from the lipids by means of size exclusion chromatography. The separated fraction was then analyzed by high temperature GC/MS. The techniques used for meat analyses were also used to study radiolytic behavior in model systems of meat components such as triglycerides or various proteins.

Electrochemical Analysis of Nitrite

Nitrite formed in irradiated frozen nitrate solutions was determined using an Orion nitrogen oxide electrode, model 95–46. Calibration curves were made for nitrite, 10^{-5} to 10^{-2} *M*, in solutions containing 10^{-3} to 10^{-1} *M* nitrate. Yields were based on linear dose responses covering an appropriate dose range to produce adequate nitrite concentrations ($\sim 10^{-4}$ *M*).

DISCUSSION

The major influences on yields may be placed into three groups that relate broadly to composition, physical state, and processing parameters. These groups are not entirely independent of each other, and some could be moved from one group to another. It is instructive, nevertheless, to consider them separately.

Compositional Factors

The amount of each food component, its distribution, and the presence of additives, including oxygen, influence the direct and indirect effects of irradiation. The amount is particularly important because it determines the partition of energy deposited in the total system, which leads to direct bond rupture and product formation. The other factors determine the secondary reactions of free radicals, if they can occur, which lead to indirect formation of other products.

Direct effects. The energy deposited in the system, strictly speaking, depends on the electron fraction of each component; but weight fraction is a satisfactory approximation for these considerations. Provided that no energy transfer occurs from a component initially absorbing the energy to another component constituting a better energy trap, as could occur in crystalline systems (6), the energy will be distributed as a linear function of the weight percent of the total that each component (or type of component) represents. The number of free radicals or molecular products formed from each component would correspond to their G-value in the pure system normalized for the fraction of the total dose deposited in that component. Consequently, the yield of a product such as CO_2 from proteins and lipids would be given by:

$$G(CO_2) = G(CO_2)_P \cdot W_P + G(CO_2)_L \cdot W_L, \quad (1)$$

where W = weight fraction, and subscripts P and L stand for protein and lipid, respectively. Similarly, the yield of product X from lipid radiolysis derived from one or more lipid components would be given by:

$$G(X)_L = G(X)_A \cdot W_A + G(X)_B \cdot W_B, \quad (2)$$

where W_A and W_B stand for the weight fraction of two such precursor components, A and B.

The distribution of the components would alter the linear dependence of yeilds on component fraction if there is significant interaction between components, particularly at interfaces, or if a minor component is inhomogeneously distributed within the larger component. Interfacial reactions between lipid and protein components, if they could occur, would be more pronounced in foods where there is extensive mixing, such as in emulsified meat products. They would be more limited if the major components were distinct phases in each of which the chemistry is independent of other phases, except at the interfaces. Reactions involving radical exchange or cross combination of radicals would take place at such interfaces and are given by:

$$P\cdot + L \rightarrow L\cdot + P \text{ (or the reverse), and} \quad (3)$$

$$P\cdot + L\cdot \rightarrow PL, \quad (4)$$

where P· and L· represent protein or lipid radicals, respectively, and PL represents the cross combination product. Such products, however, have not been identified in irradiated foods.

The influence of a component being inhomogeneously distributed primarily relates to indirect reactions (see below) and involves a concentration effect on the reaction probability. It becomes important for a component that is more soluble in one part of the medium than in another. For

example, myoglobin (Mb) represents about 1% of the wet muscle tissue, but would be concentrated in the aqueous sarcoplasma; while α-tocopherol being a fat soluble vitamin would be found in the fat portion. This consideration must be taken into account when estimating the probability of an indirect reaction involving such constituents.

The presence of additives, usually representing a small fraction of the total, is less important for direct effects than for indirect effects. Some direct decomposition of these would occur according to their weight fraction, but most effects on them, particularly the reactive additives, would be encountered in indirect processes. Additives such as ascorbic acid, phosphates, and nitrites are particularly relevant in meats. Oxygen must be considered in this context as well, since it is reactive towards free radicals and could result in objectionable product formation.

Indirect effects. Primary free radicals formed upon ionization or excitation of the component compounds can react with either the main constituents or another compound present, depending upon reactivity and concentration. Such competition for these entities if homogeneously distributed in the medium is determined by the product of reactivity, represented by the reaction rate constant, k, and the molar concentration. If solvated electrons diffuse unimpededly through the aqueous phase of a food, they might react with free amino acids (*e.g.*, glycine) or an additive such as nitrate (7), according to:

$$e^-_{aq} + {}^+NH_3CH_2COO^- \rightarrow NH_3 + {\cdot}CH_2COO^- \qquad k_5 = 8 \times 10^6 M^{-1}s^{-1} \qquad (5)$$

$$e^-_{aq} + NO_3^- \xrightarrow{H^+} NO_2^- + OH^- \qquad k_6 = 1 \times 10^{10} M^{-1}s^{-1} \qquad (6)$$

Assuming that no other reactions of electrons are possible, that the glycine concentration is $5 \times 10^{-3} M$, and that nitrate is present at $10^{-3} M$, the electron is 250 times more likely to react with nitrate than with glycine. The actual fate of the electron, however, would be determined by its reaction with the peptide bonds of proteins or with some of the more reactive free amino acids (*e.g.*, tryptophan, phenylalanine, histidine, *etc.*). This example serves to show the considerations that have to be made.

Similar considerations would be made for hydrogen atoms in the lipid component of the food. Reactions of H· in lipids primarily involve abstraction from saturated components and addition to unsaturated components. Competition by the fatty acids (8) could be envisaged as follows:

$$H{\cdot} + CH_3(CH_2)_xCH_2COOH \rightarrow H_2 + CH_3(CH_2)_x\dot{C}HCOOH \quad k_7 \sim 10^7 M^{-1}s^{-1} \qquad (7)$$

$$H{\cdot} + CH_3(CH_2)_yCH{=}CH(CH_2)_zCH_2COOH \rightarrow CH_3(CH_2)_y\dot{C}HCH_2(CH_2)_zCH_2COOH \quad k_8 \sim 10^9 M^{-1}s^{-1} \qquad (8)$$

As a consequence of the higher reactivity towards the unsaturated component, hydrogen atoms would preferentially react with this component than with the sautrated component. If initially the unsaturated fatty acids corresponds to only 1% of the saturated fatty acids or if the former are radiolysis products and build up to this level, then half of the hydrogen atoms would react with each of these two components.

Illustrative example, similarity of intermediate species. In foods that are similar in composition, similar free radicals would be formed in proportion to the weight fraction of the precursor components. Experiments in which samples of chicken, pork, beef, and ham, which have comparable proteins and fats, were similarly irradiated to 100 kGy at -80°C confirm this prediction. The actual profile of the myofibrillar proteins in these foods extracted with urea is indicated by their electrophoretic separation on polyacrylamide gels shown in Fig. 1. Major bands are due to myosin

(heavy and light chains) and actin. With the exception of a minor band appearing near the actin in chicken, these profiles are nearly identical. The fat content in the irradiated samples to be examined by electron spin resonance techniques, though not specifically determined, ranged about 10 – 15%.

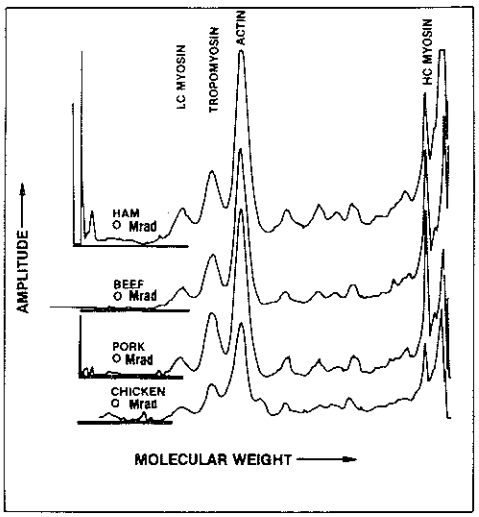


Fig. 1. Densitometric scans of polyacrylamide gels containing proteins extracted from enzyme-inactivated meat samples. Extraction was accomplished using urea and DTT. Electrophoretic separations were made using SDS. The scan for each sample is displaced vertically for clarity, and the baseline relating to each is accentuated. These samples had not been irradiated and are considered the "O Mrad" controls.

The observed ESR signals shown in Fig. 2 correspond to the collection of free radicals formed in the chicken, pork, beef, and ham and indicate the near identity for all samples. Previous work on free radicals in myosin, in beef, and in beef containing differing levels of fat support the contention that these signals are attributable to both protein and lipid free radicals (1,9,10). Slight differences in relative intensities are discernible and reflect minor differences in the fat levels. The similarity of these free radicals (the precursors of final products) in the four meats also indicates that their overall environment, which should not necessarily be identical in all cases, is not an important factor.

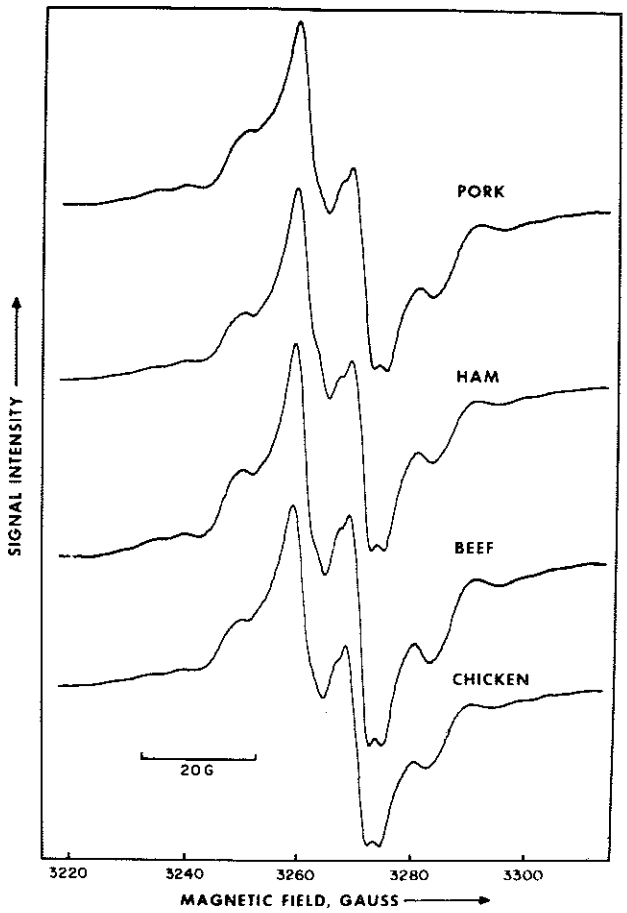


Fig. 2. Electron spin resonance spectra of finely ground samples of enzyme-inactivated meat irradiated to 100 kGy at -80°C. Samples were in the shape of cylindrical sticks and required no containers for irradiation or examination. Spectra were recorded at -80°C. Each spectrum is displaced vertically for clarity.

Illustrative example, dependence of final products. Analysis of final products of such similarly irradiated meats also confirms the dependence on component levels (11). For example, using as an indicator a lipid derived compound, octene, the influence of fat level in these four meats on lipid decomposition was examined. About a factor of two difference in fat characterized the ham, chicken, pork, and beef samples, *i.e.*, 7.3, 11.7, 14.3, and 15.4% fat, respectively. The yields of octene in parts per billion per megarad (10 kGy = 1 Mrad) of irradiation were linear with dose over the range used (0 — 120 kGy) and are shown in Fig. 3. A straight line relationship of yield with fat in the sample is clearly indicated, confirming not only that weight fraction is critical, but that the type of fat or the environment is not critical. Similar results on beef with different fat levels had been obtained previously (12,13). More examples of this approach using other indicator products, as well as products that derive from specific fatty acids in the lipids, will be presented in a forthcoming publication (11).

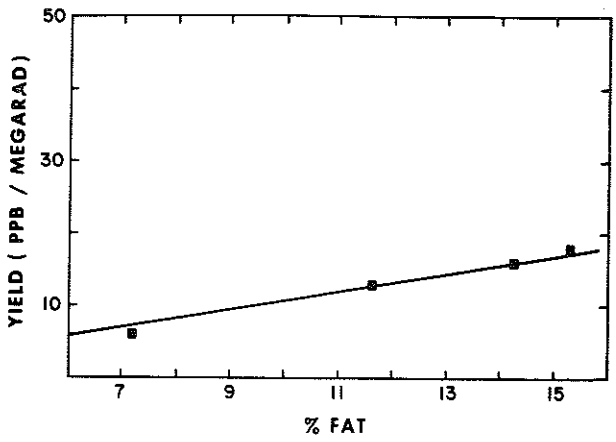


Fig. 3. Yield of octene as a function of fat content in meats irradiated at -40°C. Yields were determined from the linear increase in the amount of octene formed in each sample with increasing dose. The points plotted correspond to ham, chicken, pork, and beef samples, which have different levels of fat.

Physical State Factors

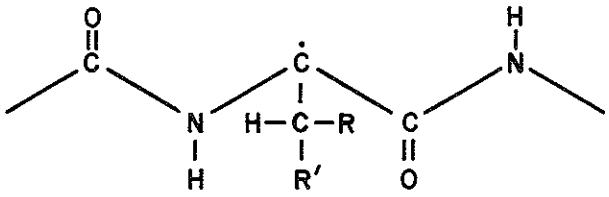
The physical nature of the food being irradiated (frozen *vs.* fluid or hydrated *vs.* desiccated) influences both the primary and secondary reactions and, consequently, determines the yields of final products. While freezing could be considered a temperature factor and hydration a compositional factor, their influences are predominantly on what happens to the intermediates rather than on what intermediates are formed.

Influence of freezing. Since the ionization and excitation of the components lead to species that must escape from the region in which they are formed to produce any net chemical effect, any factor that impedes their migration or promotes their recombination reduces the yield of products. Having a rigid ice in an irradiated food accomplishes both objectives. Ice has a long dielectric relaxation time, which promotes the return of unsolvated electrons to parent positive ions, and its high viscosity prevents hydroxyl radicals and solvated electrons from becoming uniformly distributed in the medium. The G-values for these radicals, consequently, are much lower in ice than in liquid water. For solvated electrons, the G-value (14,15) is 2.7 at 25°C and ~0.3 at -5°C. Since diffusion of these radicals is impeded, reaction with solutes would be possible only at concentrations high enough to have a solute close to a region of radical formation. Yields of products derived from electron or hydroxyl radical attack would be reduced by over 90% in ice compared to fluid solutions.

Several examples illustrating the reduction in G-values due to freezing have been published recently (16). Reduction for electron-induced NH_3 formation from glycine is ~90%; for Cl^- from chloroacetic acid, it is ~95%; and for nitrite formation from nitrate, ~99%, using $2 \times 10^{-1} M$ nitrate as a reference concentration. Indicative of the difficulty with which the solute scavenges electrons, the yield of nitrite is further reduced as the nitrate concentration is decreased. It is estimated that at $10^{-3} M$ nitrate, the G-value for nitrite would be about 0.002. The reduction in yield is

even more striking when comparing yields for hydroxyl radical-induced reactions. Yields for ferricyanide from hydroxyl oxidation of ferrocyanide are 6.1 in N_2O saturated solutions at room temperature, but only ~0.02 in 0.5 *M* ferrocyanide solutions frozen to -40°C. The practically complete elimination of indirect reactions involving water radicals when moist foods are irradiated in the frozen state has lead to the development of highly acceptable, radiation sterilized meats, despite the high doses used.

Influence of desiccation. Since the ultimate fate of radicals formed from each component depends on their ability to migrate or to twist into position for bimolecular reaction, factors that alter their environment influence their pathways for decay, and hence lifetimes. In carbohydrate and protein components, the presence of water and its involvement in the structure can significantly change the conformation of the component molecules and the disposition of the free radicals. In proteins the radical to consider (10) is the predominant peptide backbone radical:



in which the unpaired electron interacts with the hydrogen(s) on the HCRR′ group. Some indication of the effect of hydration and desiccation on radical kinetics can be gleaned from observations on the peptide radicals in two different proteins.

Decay of the peptide radical in frozen myosin is slow but perceptible (16), while its decay in desiccated gelatin at room temperature is extremely slow (17). Temperature is critical in the former case: at −40°C, the radical appears stable; at −10°C, its halflife is ~8 h; near 0°C, it is estimated to be on the order of seconds. (These radicals are short-lived in fluid, hydrated systems.) In the latter case, the radicals appear to be reduced to low levels in about 3 days, but some remain even after 1 month. The mechanism in either case is not well understood, but oxygen reaction in the gelatin samples is implicated.

Though the comparison of these two proteins is not completely valid (a better comparison would be between hydrated and desiccated samples of the same protein), it demonstrates the significance of water to the chemistry above and beyond its contribution of electrons and hydroxyl radicals. The influence of water on migration of reactive entities (*e.g.*, oxygen), as well as on molecular structure, needs to be considered.

Influence of Processing Parameters: Temperature Effects.

Yields of products will be affected by temperature if the reaction pathways are characterized by different activation energies. These activation terms could relate to barriers to reaction as well as to barriers to diffusion. Reactions of free radicals such as combination/disproportionation, addition, and electron transfer are normally diffusion-limited; radical abstraction reactions, though rapid compared to nonradical reactions, would be activation-limited. Provided that viscosity does not change appreciably within a particular temperature range, there would be only a minor dependence of the chemistry in a fluid system on temperature. A strong dependence on temperature, presumably through viscosity-like factors, are observed in frozen systems.

Illustrative of the change in yields in ice with temperature are the results for reduction of nitrate by electrons to nitrite. This dependence is shown in Fig. 4 for 2 x 10^{-1} *M* nitrate solutions irradiated over the range from −100°C to −10°C. Very little difference is observed from the lowest temperature used to about −40°C, the yield being ~0.01. A much more dramatic change is observed above −40°C, rising sharply thereafter and reaching a yield of ~0.24 at the maximum temperature used, or about one-sixth the yield observed in fluid solutions. It is not known whether the nitrate is scavenging unsolvated (or "dry") electrons (18) or solvated electrons; it may be a combination of both. In any case, the medium is clearly changing in respect to those properties that control the diffusion/migration of electrons, such as rotational relaxation times. Other factors relating to "viscosity" of medium (*e.g.*, regions of unfrozen water or supercooled water) could be having an effect. The efficacy of reaction and scavenging, consequently, is enhanced. These results are typical for many reactions observed in ices (*e.g.*, electron with chloroacetic acid, metmyoglobin, *etc.*), as well as in foods.

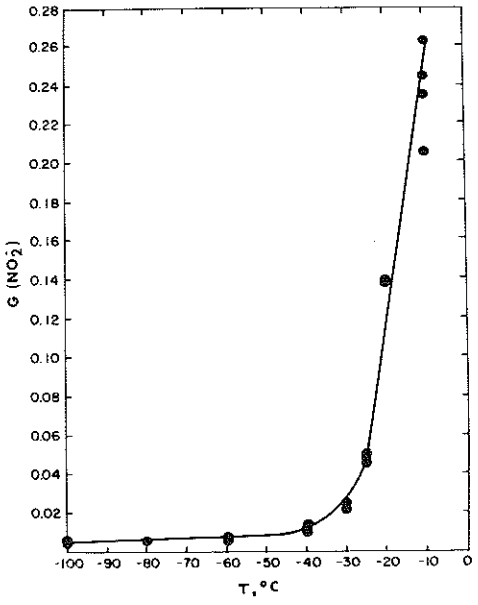


Fig. 4. Dependence of $G(NO_2^-)$ in frozen, 2 x 10^{-1} *M* nitrate solutions on temperature. $G(NO_2^-)$ is the radiation chemical yield of NO_2^- upon reduction of NO_3^- by solvated electrons. It is based on linear yield-dose plots for NO_2^- at each temperature used.

The dependence of yields from nonaqueous components also shows a marked change in the temperature range from −20°C to 0°C, judging from results on volatiles from irradiated beef. Merritt *et. al.* (12,13) have shown that lipid-derived C_5, C_6, C_7, and C_8 compounds increase slightly from −80°C to −20°C, and then markedly above −20°C. Viscosity of the lipids changes in this region so that the increased yields reflect either the greater probability of escape of radicals from "cages" or the greater efficiency of those molecular or primary free radical reactions responsible for the formation of such radicals.

Influence of Processing Parameters: Dose-Rate Effects.

Provided that certain conditions are met, the dose-rate influences the course of competitive pathways for reaction and determines the relative yields of products of those reactions. One important condition is that the reactive entities be uniformly distributed; another is that there be a competition between second-order (or higher) and first-order reactions involving the same intermediate. The effects, if they occur, would be discernible when comparing the high dose-rates associated with machine sources of irradiation compared to the low dose-rates commonly encountered with radioisotope sources. The dose-rate of the former could easily be 10^6 times as high as the latter. The presence of other reactive constituents, additives, or radiolysis products will have an influence as well.

Inhomogeneous decay. Uniformity of distribution of free radicals depends on the nature and viscosity of the medium. Initially, these reactive entities are clustered in spurs, which are separated along the trajectory of the ionizing particle, the interspur distances being determined by the linear energy transfer (LET) value of the particle. (These distances would be short for alpha particles, longer and approximately the same for electrons and gamma rays.) Reactions, such as recombination and neutralization, take place in the spurs at the same time diffusion out of the spurs occurs. If the temperature is low or the viscosity is high, this diffusion is impeded or is entirely precluded. In either case, the distribution remains relatively inhomogeneous. Reaction then takes place among entities of the same spur, and the reaction rate would be determined by the separation distance of the "spatially correlated" partners. Those close to each other react more rapidly than those further apart. Since increased dose-rate would alter the number of spurs and not the separation distances (except where spur overlap is encountered), there would be no dose-rate effect.

Illustrative of the decay of inhomogeneously distributed radicals is the reaction of electrons in a viscous hydrocarbon such as squalane (19). The electrons were generated in this system pulse radiolytically at -140°C, and their decay monitored optically at 1600 nm. The reaction does not follow simple kinetics laws, and the concentration-time profile is characterized by a sharp drop followed by a long tail. Most importantly, for systems in which the initial electron concentration differed by a factor of 30, the halflife of decay was the same. Normalizing the concentration in terms of "surviving fraction" showed that the curves for different initial concentrations could be superimposed. As expected, the increase in the number of spurs or reactive entities merely increases the number reacting at a specific rate independent of the presence of other entities.

Homogeneous decay. If the reactive entities are uniformly distributed and straightforward kinetics laws apply, then it is the instantaneous (or steady-state) concentration of the radical involved in the second-order and first-order (or psuedo-first-order) reactions that determines its fate. For an idealized system in which hydrogen atoms are generated rapidly and undergo the following reactions:

$$H\cdot + H\cdot \rightarrow H_2 \qquad k_9 = 2 \times 10^{10} M^{-1} s^{-1} \tag{9}$$

$$H\cdot + RCH_2CH{=}CHCH_3 \rightarrow RCH_2\dot{C}HCH_2CH_3 \quad k_{10} \sim 10^9 M^{-1} s^{-1}, \tag{10}$$

the relative rate of hydrogen loss by reaction 9 compared to reaction 10 would be given by equation 11:

$$\begin{aligned}(-d[H\cdot]/dt)_9 \;/\; (-d[H\cdot]/dt)_{10} &= k_9[H\cdot]^2/k_{10}[H\cdot][RCH_2CH{=}CHCH_3] \\ &= k_9[H\cdot]/k'_{10}\end{aligned} \tag{11}$$

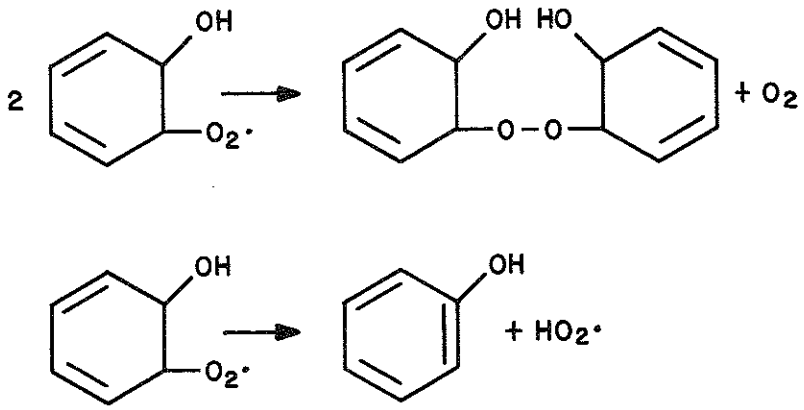
Since the alkene is considered to be in large excess compared to H·, its concentration is constant and the psuedo-first-order rate constant, k'_{10}, which includes $[RCH_2CH{=}CHCH_3]$, can be used. At

an instantaneous H· concentration of $10^{-6}M$ in this system with $[RCH_2CH{=}CHCH_3] = 10^{-4}M$, five times as many H· react with the alkene than with each other; at $[H\cdot] = 10^{-8}M$, essentially all react with the alkene. Assuming that no steady-state concentration of H· is achieved, then [H·] is determined by the time of irradiation (pulse length), the G-value for H· formation, and the dose-rate (dD/dt):

$$[H\cdot]_t = \int_0^t (d[H\cdot]/dt)dt \propto G(H\cdot) \int_0^t (dD/dt)dt \qquad (12)$$

If the irradiation times are long, as is the case for gamma irradiation, a steady-state concentration of H· is obtained that would be reflected in the relevant mathematical expressions for the kinetics, expressions in which dose-rate would appear explicity. (Discussion of this case will not be included here.)

A study of phenol formation in irradiated, aerated solutions of benzene (a model system that is compositionally unrelated to food) provides a simple illustration of the dose-rate effect. Dorfman and co-workers (20) used the results to substantiate the mechanism suggested to explain the decay of the intermediate formed upon OH· addition to benzene and subsequent reaction with O_2. This intermediate decays bimolecularly and unimolecularly according to the following scheme:



By varying the dose per pulse, they could change the concentration of the intermediate peroxy radical that is obtained at the end of the pulse, and alter the competition between reactions 13 and 14. Consequently, at low doses per pulse, the phenol yield was found to be ~2; while at higher doses per pulse, the yield dropped to ~0.3, indicating the predominance of reaction 13.

In systems as complex as food, dose-rate effects involving primary radicals are not expected to be significant, because psuedo-first-order reactions with the main components will predominate at almost all dose-rates used in practice. Reactions of e^-_{aq}, H·, and OH· with the proteins and carbohydrates and of H· with lipids are favored because of the large "concentration" of these components, despite their low reactivity in some cases. Only a minor amount of combination of these radicals would take place. (Moreover, if the system is frozen, these radicals do not distribute uniformly, and inhomogeneous kinetics prevails.) Consequently, secondary radicals will be formed, some of which in proteins and lipids will preferentially convert by psuedo-first-order (or true first-order) processes to more stable radicals. Since these secondary or tertiary radicals will react exclusively by bimolecular, termination reactions, there should be little or no dose-rate effect discernible, *i.e.*, there should be no difference in yields between electron and gamma irradiation. Studies on radiation sterilized meats using both sources confirm this expectation (12).

Influence of Processing Parameters: Dose Effects

Increasing the irradiation dose should increase the amount of each product but should not alter the spectrum of products. This linear dependence of yields would be observed for low doses; deviations from linearity would be encountered only at higher doses where secondary reactions might be introduced. The theoretical basis for specific dependences of yields on dose have been discussed elsewhere in connection with the validity of extrapolating conclusions on the wholesomeness of foods from high doses to low doses (21). It had been shown that for primary products the three major yield-dose profiles — linear, plateau, and peaked — could be embodied in an idealized reaction scheme involving a major component, a minor component, a reactive intermediate, and a few stable products. When doses are reached at which primary products are affected, secondary products would be discernible. The factors determining these product profiles are considered here briefly.

Yield-dose dependences. If a major component upon irradiation gives rise to products M, A, and B and an intermediate X by direct action,

$$S \xrightarrow{\gamma} M,\ A,\ B,\ X\ \ldots, \tag{15}$$

then, providing that no other reactions involve A, its yield will be linear. If B builds up in concentration so as to compete with S for X,

$$S + X \rightarrow M \tag{16}$$

$$B + X \rightarrow D \tag{17}$$

then eventually as much B is lost as is formed and a plateau in its concentration is reached. The profiles for A, B, and D are shown in Fig. 5a. (Some deviation in linearly for M would also be seen.) If a minor component, S′, reacts with X to produce E, a compound that is also reactive to X,

$$S' + X \rightarrow E \tag{18}$$

$$E + X \rightarrow F \tag{19}$$

then E is removed without being replenished because S′ is depleted, so its concentration reaches a maximum. The profiles for S′, E, and F are shown in Fig. 5b.

Implication for food. For deviations from linearity to occur, products such as B or E must be extremely reactive towards electrons or H· and must be formed with reasonably high G-values. If S is for proteins or lipids, which are reasonably reactive and in huge concentration, then $k_{17}[B]$ must be larger than $k_{16}[S]$ for B to compete effectively with S for the intermediate. For example, an alkene formed in the fat must reach a concentration of about $5\times10^{-3}M$ to compete with a saturated substrate for H·. A dose in excess of 250 kGy is required to produce this concentration if the G(alkene) were ~0.2. (The kinetics considerations become more complicated if H· reacts with other, less mobile intermediates that might build up in the system.) In general, extremely high doses would be needed before secondary reactions are encountered, and results for foods show linear dependences of product formation on dose.

The significance of the linear yield-dose response to the wholesomeness of irradiated foods is that the safety data obtained on systems receiving high doses can be applied to similar systems receiving low doses. For both cases, the same spectrum of radiolysis products is to be evaluated, albeit at different levels. Consequently, if toxicological procedures indicate no adverse effects upon consuming a food irradiated to 50 kGy, then that food must be equally innocuous at 5 kGy.

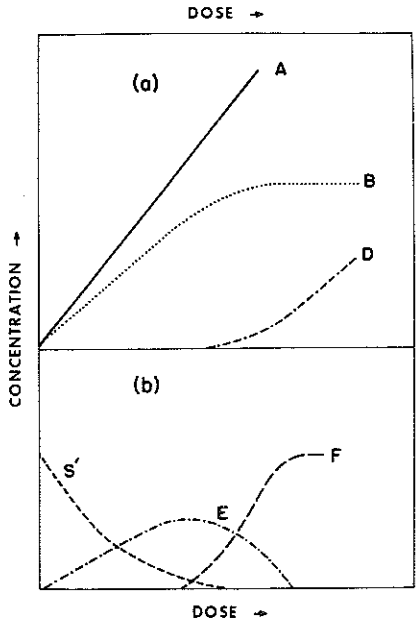


Fig. 5. Idealized representation of the different concentration-dose profiles for products in an irradiated food. The scales for concentration and dose are arbitrarily set. (a) Profiles for primary products A and B and secondary product D (see text). (b) Profiles for minor component of the food, S', and products E and F. The dose scales for (a) and (b) are not necessarily coincident (see text).

As described previously, results on irradiated haddock illustrate this principle of extrapolation (21). Wholesomeness studies were conducted on haddock (and codfish) irradiated to 2, 6, 28, and 56 kGy that proved their safety. Chemical studies showed that yields of volatiles increased linearly with dose, at least up to 10 kGy. It can be assumed, therefore, that extrapolating down to lower doses, including 1 kGy, is valid. This dose was of interest for irradiating ocean fish on board ship, and health authorities questioned whether existing data are applicable (22).

CONCLUSION

From the considerations made and illustrative data presented, several conclusions can be reached relevant to clearing irradiated foods:

(1) The major radiolysis products and their approximate yields can be predicted on the basis of the chemistry of the components, their weight fractions in the food, and the irradiation conditions.

(2) Foods similar in composition and in radiolytic response for a basic set of irradiation conditions should be taken as a generic group and viewed as one test substance in wholesomeness studies.

(3) Health authorities should be able to grant clearances for generic foods or to extend an existing clearance on one member of the group to other foods in the group – on the basis of chemical data and related toxicological and microbiological tests, without requiring extensive animal feeding studies.

All of these principles and the supporting chemical data are going to be reviewed by the FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food when they meet again in 1981 to consider granting broad approvals of food irradiated up to a dose of 10 kGy (23).

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